REMARKS

Reconsideration and withdrawal of the claim rejections are requested in view of the amendments and remarks herein.

I. STATUS OF CLAIMS AND FORMAL MATTERS

Claims 21-41 are pending in this application. Claims 21, 22, 27, 31 and 34-40 have been amended, and claim 41 has been added.

Support for the amendments can be found throughout the specification. The majority of the amendments are made for clarity and to place the claims in better form. No new matter is added.

It is submitted that the claims, herewith and as originally presented, are patentably distinct over the prior art, and that these claims are and were in full compliance with the requirements of 35 U.S.C. §112. The amendments of and additions to the claims, as presented herein, are not made for purposes of patentability within the meaning of 35 U.S.C. §§§§101, 102, 103 or 112. Rather, these amendments and additions are made simply for clarification and to round out the scope of protection to which Applicants are entitled. Furthermore, it is explicitly stated that the herewith amendments should not give rise to any estoppel, as the herewith amendments are not narrowing amendments.

In response to the Notice of Draftsperson's Patent Drawing Review, Figures 1, 4 and 5 are submitted herewith. It is believed that the margins of Figure 1 fall within the required dimensions. In addition, the shading in Figures 4 and 5 has been removed. Acceptance of the drawings is requested.

II. THE REJECTIONS UNDER 35 U.S.C. §112, 1ST PARAGRAPH ARE OVERCOME

Claims 27 and 31 were rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. Claims 32, 33 and 40 were also rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking enablement. These rejections are traversed, and will be addressed collectively.

The Office Action maintains that the only disclosed use for the invention is gene therapy, and alleges that the specification is enabling for methods of delivering nucleotide sequences to neuronal cells *in vitro*, but not *in vivo*. The Office Action goes on to state that the Declaration under 37 CFR 1.132 by Drs. Wong and Mazarakis is insufficient to overcome the rejection because the vector and anti-apoptotic molecule, Bcl, which were used in the experiments

described in the Declaration were not disclosed in the application, and because there is allegedly no evidence that the rat stroke model is art recognized as being predictive of results expected in humans.

The instant invention pertains to the use of rabies G protein for pseudotyping retroviral vectors, and the use of the pseudotyped retroviral vectors for efficient transduction of neuronal cells and delivery of genes of interest. The specification teaches such pseudotyping in the context of a retroviral vector, and in particular, uses examples with MLV, HIV, and EIAV retroviral vectors. The specification teaches a surprising result with respect to the use of rabies G for pseudotyping retroviral vectors, as compared to the use of VSV-G for pseudotyping, in the context of superior transduction efficiency in neuronal cells. It is noted, with emphasis, that the results disclosed in the specification are the result of the rabies G pseudotyping technology, and not the result of optimization of the retroviral vector to be pseudotyped. Optimization parameters were available in the state of the art, with respect to generating retroviral vectors with an enhanced safety profile. Such optimization included generating retroviral vectors having very few or no accessory genes and/or modified LTRs, so that the generation of replication competent virus could be averted. For example, Kim *et al.* (Journal of Virology, Jan 1998, 72(1): 811-816; copy enclosed) set forth optimization of a lentiviral genome by removal of accessory genes and deletion of LTR sequences. These vectors are also reported in Examples 1 and 2, in Table 1, and in Example 8, pages 41-42, of the specification. Accordingly, the specification teaches by example, and further describes, that retroviral vector genomes preferably contain a minimum of retroviral structural components. (See page 22, lines 8-10, of the specification.)

The Office Action asserts, on pages 3 and 4, that the vector used in the Wong and Mazarakis Declaration is not disclosed in the specification; and therefore, that its characteristics cannot be deemed to be related to the transduction and neuroprotection of rat neuronal cells in the rat stroke model. The Declaration discusses the EIAV pONY8Z/rabies vector, a control vector expressing β-gal and the EIAV pONY8.Bcl2/rabies vector, which is a therapeutic vector expressing the anti-apoptotic molecule Bcl2. The specification discloses the EIAV genomic vector pONY2.1, from which the further optimized vector backbone pONY8 is derived.

As optimization parameters were available to the ordinarily skilled artisan, and are further provided as a guideline in the specification, optimization of the retroviral vectors is fully enabled by the specification. The pONY2.1 vector is one of the vector derivatives in the pONY

series of EIAV vectors. pONY2.1 includes a partial deletion of *gag*; pONY4 further includes a deletion to make the *tat*, *S2*, *env*, and *rev* genes nonfunctional; and, pONY8 includes further deletions in the *gag*, *tat*, *S2*, and *rev* genes. All deletions are the result of further optimization of the vectors set forth in the specification.

This EIAV vector information is found in GB9727135.7 (priority document of WO99/32646; copy enclosed), which is incorporated in the specification by reference on page 27. Furthermore, the vector information is available in the public domain in Mitrophanous *et al.* (Gene Therapy (1999) 6: 1808-1818; copy enclosed) and in Mazarakis *et al.* (Human Molecular Genetics (2001) 10(19): 2109-2121; copy enclosed). In fact, Mazarakis *et al.* reported that retroviral vectors pseudotyped with rabies G led to enhanced gene transfer *in vitro* and *in vivo*, *e.g.*, after injection in rat brain, spinal cord, or muscle, as compared to vectors pseudotyped with VSV-G. Such results are consistent with and obtainable by the teachings of the specification.

The retroviral vector system of the invention is capable of expressing one or more nucleotide sequences of interest (NOIs). The specification discloses that such NOIs may be any one or more of selection gene(s), marker gene(s), and/or therapeutic gene(s). (See page 19, lines 14-30.) Non-limiting examples of therapeutic NOIs and diseases to be treated are set forth on page 20 of the specification. As such, the retroviral vector system pseudotyped with rabies G is a vector system to be used to deliver <u>any</u> particular NOI(s) for treatment of a corresponding disease or diseases. The rat stroke model set forth in the Wong and Mazarakis Declaration is just one such example: a therapeutic NOI encoding Bcl2 is used for the treatment of a corresponding disease in a model system. In this instance, the disease is indicative of glutamate-induced neuron damage, which is a well-established hallmark of ischaemic cerebral stroke. While the Bcl-2 molecule is responsible for the therapeutic action of neuroprotection, the retroviral vector system pseudotyped with rabies G is the tool that makes it possible to efficiently transduce a high number of neurons in the hippocampus. This observation is supported by Mazarakis *et al.*, *supra,* who report efficient transduction of neurons in the rat brain by measuring lacZ expression. In fact, the Applicants have recently achieved therapeutic results using the rabies G pseudotyped retroviral vectors of the instant invention in mouse models for amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA). These results are not yet published, but are mentioned here by way of example that a therapeutic gene may be selected based on a

series of EIAV vectors. pONY2.1 includes a partial deletion of *gag*; pONY4 further includes a deletion to make the *tat*, *S2*, *env*, and *rev* genes nonfunctional; and, pONY8 includes further deletions in the *gag*, *tat*, *S2*, and *rev* genes. All deletions are the result of further optimization of the vectors set forth in the specification.

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corresponding disease to be treated. Such selection is well within the purview of the skilled artisan.

In a mouse model for ALS, transgenic SOD-1 mice typically survive an average of 125-130 days, and, as is the case with ALS patients, die from diaphragm muscle failure. Applicants were able to use the rabies G pseudotyped retroviral vector of the instant invention to deliver a neuroprotective gene to the diaphragm muscle to target diaphragm motor neurons. As a result, the survival of the treated SOD-1 mice was prolonged, as compared to that of the control mice.

In a mouse model for SMA, transgenic mice lacking the endogenous gene encoding the survival of motor neuron protein (SMN), but carrying copies of human SMN2, represent an animal model of type 1 (severe) SMA; these mice die at 12-14 days of age. Muscular atrophy is due to degeneration of motor neurons in the spinal cord. Applicants administered the rabiesG pseudotyped retroviral vector carrying the gene encoding SMN via the major muscle groups of the mice, thereby transducing the motor neurons projecting into the muscle, and, as a result prolonging survival of the treated mice, as compared to untreated, control mice.

It should be noted that the foregoing experiments and results are described in Examples 16 and 17 of co-pending U.S. application Serial No. 10/716,725, filed on November 19, 2003. That application is directed to methods of using the invention claimed in this application. The Examiner is invited to refer to U.S.S.N. 10/716,725 for the details of the above-described studies.

With respect to the rat model for ischaemic stroke that was discussed in the Wong and Mazarakis Declaration, Applicants have relied upon their skill in the art, combined with the state of the art, to produce a model that exhibits the well-established excitotoxicity characteristic associated with cerebral ischemia (stroke).

Cerebral ischaemia leads to severe brain injury that is often a result of complex pathophysiological events including excitotoxicity, periinfarct depolarizations, inflammation and programmed cell death. One major factor in initiating ischaemic cell death is the activation of glutamate receptors such as NMDA and AMPA/kainate receptors, which are present on CNS neurons. Neuronal loss occurs due to excitotoxicity, a feature whereby excessive activation of glutamate receptors triggers cell death.

During ischaemic stroke, the transient loss of cerebral blood flow impairs the energetics required to maintain ionic gradients (Martin *et al.* (1994) Trends Neurosci 17: 251-257; Abstract enclosed), causing depolarization and the release of excitatory amino acids into the extracellular

space. The high concentration of glutamate activates NMDA and metabotropic glutamate receptors, and triggers a massive influx of Na^+ and Ca^{2+}. (See Choi *et al.* (1990) Annu Rev Neurosci. 13:171-82; and Choi (1995) Trends Neurosci. 18:58-60.) Brain oedema ensues and Ca^{2+}, via its universal role as a second messenger, initiates a series of cytoplasmic and nuclear events that cause tissue damage. These downstream cascades that eventually lead to cell death are mediated by a variety of necrotic and apoptotic mechanisms (Dirnagl *et al.* (1999) Trends Neurosci. 18:58-60; Abstract enclosed), for example, the generation of free-radicals, disruption of mitochondrial activity (Dugan *et al.* (1994) Ann Neurol 35: S17-20; Abstract enclosed) and activation of caspases. The intracellular signalling pathways activated during excitotoxicity can also trigger the expression of genes that initiate post-ischaemic inflammation, which can then further contribute to the pathogenicity of ischaemia.

As one of the key events in the pathology of ischaemic stroke is the activation of glutamate receptors, one therapeutic approach has been to block receptors that are activated by glutamate, for example the use of MK801, an NMDA receptor antagonist (Prass *et al.* Restor Neurol Neurosci 13: 3-10; Abstract enclosed). The NMDA receptor controls an ion channel that is permeable to Ca^{2+}, Na^+ and K^+. These observations support that an excitotoxicity model based on the direct injection of lethal doses of NMDA into the brain is correlative with injury that occurs after an ischaemic stroke. In transient global ischaemia, the delayed neuronal death has been observed in selectively vulnerable brain regions such as the hippocampal CA1 region and caudate putamen (Pulsinelli (1992) Lancet. 339:533-6). Therefore, selectively injecting the hippocampus with an excitotoxic agent, *e.g.*, NMDA, causes neurons in the CA1 region of the hippocampus to undergo neurodegeneration, with the appearance of pyknotic nuclei and expression of activated caspase-3, features that have been observed in ischaemic stroke.

Many studies have reported the use of the middle cerebral occlusion (MCAO) and 4-vessel occlusion (4-VO) models, which provide good animal models of ischaemic stroke. However, in these models, damage produced can be variable from one animal to the next; therefore a large number of animals need to be tested in order to reach a reliable conclusion. To the contrary, the excitotoxicity model disclosed in the Wong and Mazarakis Declaration is based on the scientific principles and pathology of ischaemic stroke, and offers a more consistent model of neuronal damage reminiscent of ischaemic injury, and as the art recognizes. This not

only minimizes the amount of animal experimentation, it also provides a good model for evaluating the neuroprotective functions of putative genes.

Regardless, the rat stroke model of the Declaration is not the instant invention *per se*. The model merely provides an example, in a therapeutic setting, of the claimed retroviral vector system pseudotyped with rabies G, functioning as taught and described in the specification, with respect to efficient neuronal transduction. Further evidence that the retroviral vector system of the invention functions as described is found in the Applicants' own work, Mazarakis *et al. supra*. To that end, the instant invention is a tool for gene delivery, in both a therapeutic setting and in a research setting, both *in vitro* and *in vivo*. Therefore, the claimed invention is fully enabled by the teachings of the specification.

Pages 4-5 of the Office Action refer to safety considerations and the difficulty of relating research and animal studies to successful human clinical trials. However, as was pointed out in the previously filed Amendment, considerations made by the FDA for approving clinical trials are very different from those made by the PTO in determining whether a claim is enabled. In other words, safety considerations are more properly left with the FDA. *Scott v. Finney*, 34 F.3d 1058, 1063, 32 USPQ2d 1115, 1120 (Fed Cir. 1994). It is reiterated that the claimed invention pertains to a novel retroviral vector delivery system to deliver a gene of interest to a target cell. The specification fully enables the claimed invention in both *in vitro* and *in vivo* contexts, and the scope of protection of the claims should properly reflect the same.

Claims 21-33 and 40 were rejected under 35 U.S.C. §112, first paragraph, as allegedly lacking adequate written description. The Office Action states that sufficient description does not exist for the genus of retroviral vectors comprising derivatives of the rabies G protein because such derivatives do not necessarily need to have any structural relationship with the rabies G protein. The term "derivative" has been removed from the claims, obviating this rejection.

It is submitted that the requirements of 35 U.S.C. §112, first paragraph, have been met. Therefore, reconsideration and withdrawal of those rejections are requested.

III. THE REJECTIONS UNDER 35 U.S.C. §112, 2ND PARAGRAPH ARE OVERCOME

Claims 21-39 were rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite. The rejections are traversed.

Claims 21, 34-39, and dependent claims were allegedly indefinite due to their recitation of "selectively transducing human target cells with higher transduction efficiencies in neuronal cells". The Office Action alleges that it is unclear which target cells are selectively transduced. The word "human" has been removed from the claims. It should now be clear from the claim language and from the record that the higher transduction efficiencies are achieved using a retroviral vector delivery system that is pseudotyped with rabies G protein than are achieved using a retroviral vector delivery system pseudotyped with a VSV-G protein.

Claims 21-33 and 40 were allegedly indefinite in reciting a derivative of a rabies G protein. As discussed above, this recitation has been deleted from the claims, rendering the rejection on this basis moot.

Consequently, reconsideration and withdrawal of the rejections under 35 U.S.C. §112, second paragraph, are requested.

IV. THE OBJECTIONS UNDER 37 C.F.R. §1.75(c) ARE OVERCOME

Claims 23 and 24 were objected to as allegedly being of improper dependent form for failing to further limit the subject matter of a previous claim. The amendment to independent claim 21 obviates the objection; therefore, reconsideration and withdrawal are requested.

CONCLUSION

In view of the amendments and remarks herewith, the application is believed to be in condition for allowance. Favorable reconsideration of the application and prompt issuance of a Notice of Allowance are earnestly solicited.

Respectfully submitted,

FROMMER LAWRENCE & HAUG LLP
Attorneys For Applicants

By: Anne-Marie C. Yvon

Thomas J. Kowalski
Reg. No. 32,147

Anne-Marie C. Yvon, Ph.D.
Reg. No. 52,390
Tel. (212) 588-0800